

Certificate of Analysis for NR-30877

Mycobacterium tuberculosis, Strain 98-2766

Catalog No. NR-30877

This reagent is the tangible property of the U.S. Government.

Product Description:

Mycobacterium tuberculosis (M. tuberculosis), strain 98-2766 was isolated between 1995 and 2000 from human sputum from an HIV-negative patient infected with pulmonary tuberculosis in North America.

Lot: 70003472¹ Manufacturing Date: 07JUL2017

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ² Cellular morphology	Crom positive reds	Cram positivo rada
Colony morphology ³	Gram-positive rods Report results	Gram-positive rods Irregular, slight peaked, undulate,
	Report results	rough and cream (Figure 1)
Growth rate	> 7 days	22 days
Growth at 26°C	≥ 7 days	
	Negative Positive	Negative Positive
Growth at 37°C		
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Pigmentation in the dark (Scotochromogen)	Negative (no pigment)	Negative (no pigment)
Photoinduction for 1 hour (Photochromogen)	Negative (no pigment)	Negative (no pigment)
Nonchromogen (no pigment)	Positive (no pigment)	Positive (no pigment)
Biochemical tests		
Niacin production ⁴	Positive	Positive
Nitrate reduction	Positive	Positive
Pyrazinamidase	Positive	Positive
Antibiotic Susceptibility Profile		
Sensititre™ System ^{5,6}		
Amikacin	Report results	0.25 μg/mL
Cycloserine	Report results	16 μg/mL ^{7,8}
Ethambutol	Report results	1 μg/mL ⁸⁻¹⁰
Ethionamide	Report results	1.2 μg/mL ¹⁰
Isoniazid	Report results	≤ 0.03 µg/mL
Kanamycin	Report results	2.5 μg/mL
Moxifloxacin	Report results	0.25 μg/mL
Ofloxacin	Report results	1 μg/mL
Para-aminosalicylic acid	Report results	≤ 0.5 µg/mL ¹⁰
Rifabutin	Report results	≤ 0.12 µg/mL ¹⁰
Rifampin	Report results	≤ 0.12 µg/mL
Streptomycin	Report results	0.5 μg/mL ¹⁰
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene	≥ 99% sequence identity to	100% sequence identity to
(~ 400 base pairs)	M. tuberculosis type strain	M. tuberculosis type strain
	(GenBank: AL123456)	(GenBank: AL123456) ¹¹
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment ¹²	Growth consistent with expected	Growth consistent with expected
	colony morphology	colony morphology
Tryptic Soy agar ¹³	Report results	Growth consistent with expected
,, , - 	f	colony morphology
Viability (post-freeze) ³	Growth	Growth
(ND 00077 was a residual desired and the second the sec	GIOWIII	GIOWIII

¹NR-30877 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 46 days at 37°C in an aerobic atmosphere with 5% CO₂. The resulting growth was harvested in Middlebrook 7H9 broth with ADC enrichment supplemented with 10% glycerol and frozen. The frozen material was later thawed and aliquoted into cryovials and frozen to produce this lot.

BEI Resources

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²Information on *Mycobacterium* testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria." <u>Biochemical Testing.</u> (2012) Jose C. Jimenez-Lopez (Ed.), InTech, http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." <a href="https://link.growing.nc.nlm.nlm.growing.nlm.grow

³22 days at 37°C in an aerobic atmosphere with 5% CO₂ on Middlebrook 7H10 agar with OADC enrichment

⁴All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.

⁵Sensititre™ System *Mycobacterium tuberculosis* MIC Plate, Thermo Scientific™, catalog number MYCOTB

⁶Minimum Inhibitory Concentration (MIC); No Clinical & Laboratory Standards Institute (CLSI) interpretations of the Sensititre™ System data for *M. tuberculosis* are currently available.

⁷Two MICs were observed for cycloserine (8 μg/mL and 16 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.
⁸Variability in the MIC result by the Sensititre[™] method has been demonstrated (Lee, J., et al. "Sensititre MYCOTB MIC Plate for Testing Mycobacterium tuberculosis Susceptibility to First- and Second-Line Drugs." Antimicrob. Agents Chemother. 58 (2014): 11-18. PubMed: 24100497.), with the results for a single antibiotic typically within one doubling dilution.

⁹Two MICs were observed for ethambutol (≤ 0.5 μg/mL and 1 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.

¹⁰For ethambutol, ethionamide, para-aminosalicylic acid, rifabutin and streptomycin, the endpoint for these drugs is determined by the well with approximately 80% inhibition of growth compared to the positive control well with no drug.

¹¹Also consistent with M. africanum, M. bovis, M. canettii, M. caprae and M. microti

¹²Purity of this lot was assessed for 53 days at 37°C in an aerobic atmosphere with 5% CO₂.

¹³Purity of this lot was assessed for 22 days at 37°C in an aerobic atmosphere with 5% CO₂.



Figure 1: Colony Morphology

/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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